*Glycoconjugate Journal* (1996) **13:** 1021–1029

# Elevation of an $\alpha$(1,3)fucosyltransferase activity correlated with apoptosis in the human colon adenocarcinoma cell line, HT-29

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Received 30 November 1995, revised 23 February 1996

We studied changes in the carbohydrate expression following apoptotic cell death induced by treatment with interferon (IFN)-$\gamma$ and anti-Fas antibody using human colon adenocarcinoma HT-29 cells. An apoptotic cell death of HT-29 accompanied with typical DNA fragmentation was observed when the cells were cultured sequentially with IFN-$\gamma$ and anti-Fas antibody. In flow cytometric analyses, the expression of $Le^x$ and $Le^y$ antigen was strongly and slightly enhanced, respectively, on the cell surface in accordance with the apoptosis. When the fucosyltransferase (Fuc-T) activities of the lysates from the treated cells were examined relative to those from untreated cells, a 2.5-fold increase of $\alpha$(1,3)-Fuc-T activities and a slight increase of $\alpha$(1,2)-Fuc-T activities were observed, but little or no increase of $\alpha$(1,4)-Fuc-T activity was detected. In Northern blot analyses using probes for Fuc-T III, IV, V, VI and VII genes, strong RNA messages for Fuc-T III, V and/or VI and a weak RNA message for Fuc-T IV were detected in the untreated HT-29 cells. On the other hand, in the treated cells, the messages for Fuc-T III, V and/or VI were found to almost disappear and the 2.3 kb message for Fuc-T IV was observed to elevate 2.8-fold. Therefore, we suggest that the strongly increased expression of $Le^x$ antigen found on the HT-29 cell surface might be involved in the process of apoptosis, and that the enhancement of the antigen expression seems to result from the increased activity of $\alpha$(1,3)-Fuc-T encoded mainly by the Fuc-T IV gene.

*Keywords*: apoptosis, fucosylated carbohydrate antigens, fucosyltransferases, FACS analysis, Northern blot, RT-PCR

*Abbreviations*: Bn, Benzyl; EDTA, ethylenediaminetetraacetic acid (sodium salt); SDS, sodium dodecyl sulfate; D-PBS, Dulbecco's phosphate buffered saline (metal free); FITC, fluorescein isothiocyanate; AIDS, acquired immune deficiency syndrome; FACS, fluorescence-activated cell sorter; IFN, interferon; dNTP, deoxynucleosides-triphosphate; PCR, polymerase chain reaction; kb, kilobase(s); bp, base pair(s).

## Introduction

Apoptosis (programmed cell death) is a basic physiological process for the elimination of specific types of cells, and is known to play a key role in several cell processes as follows: development and morphogenesis, determination of the size and shape of development of tissues, maintenance of the dynamic steady state in turnover of certain cell lineages in normal tissues, and definition of the balance of tumour cell populations [1–4]. It has been demonstrated that the stimulation of the Fas antigen on the cell surface by anti-Fas antibody leads the cell into the process toward apoptotic cell death in some types of cells

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and that the achievement of apoptosis is confirmed by morphological changes and DNA fragmentation [5, 6]. Recently, it has been found that some oncogenes such as *bcl*-2 and c-*myc* are associated with apoptosis. The apoptosis of the cells was suppressed by the expression of *bcl*-2 antigen in the cell, but was enhanced by that of c-*myc* [7, 8]. The apoptosis might help to define the balance of tumour cell populations since apoptotic cells have often been found in tumour tissues [1–3]. Thus, apoptotic cell death seems to be controlled by some oncogenes which are associated with cell growth and cell differentiation, and the apoptotic process seems to play an integral role in oncogenic progression.

It was recently demonstrated that the appearance of cell surface glycosphingolipids, such as globotriaosylceramide, in the tonsillar B lymphocyte germinal centre was closely associated with B cell apoptosis [9]. More recently, it was shown that the binding of verotoxin to the carbohydrate moiety induces cell death in $Gb_3/CD77^+$ Burkitt's lymphoma cells [10]. In an immunohistochemical study, $Le^y$ antigen expression was reported to be closely related with apoptosis [11]. However, these reports have only been focused on the expression of carbohydrate antigens associated with apoptosis, and the enzymatic and molecular genetic backgrounds were not studied.

The alteration in the expression of glycoconjugates has been observed in a variety of cancers, and the presence of aberrant glycoproteins and glycolipids, which are absent or minimal in normal tissues, has been found in various cancer tissues [12–14]. These tumour-associated carbohydrate antigens, in particular, the antigens belonging to the type 1 [Gal$\beta$1,3GlcNAc$\beta$] and the type 2 [Gal$\beta$1,4GlcNAc$\beta$] chains, have been shown to be developmental antigens and differentiation markers, and they have been suggested to be involved in cell-to-cell interaction during ontogenesis and organogenesis [15]. It is known that HT-29 colon adenocarcinoma cells undergo the process of apoptosis by sequential treatments with IFN-$\gamma$ and anti-Fas antibody [5]. In the present study, we investigated not only the changes of both the expression of the carbohydrate antigens possessing type 1 and type 2 chains and the activity of $\alpha$(1,2)-, $\alpha$(1,3)- and $\alpha$(1,4)-Fuc-Ts but also the message levels of $\alpha$(1,3)-Fuc-T genes in the process of apoptosis of human adenocarcinoma HT-29 cells.

## Materials and methods

### *Cell lines and monoclonal antibodies*

The human colon adenocarcinoma cell line HT-29, human epidermal carcinoma cell line (A431) and human promyelocytic leukaemia cell line (HL-60) were obtained from American Type Culture Collection and cultured in a $CO_2$ incubator at 37 °C with the original culture medium.

Monoclonal antibodies against carbohydrate antigens used in this study were listed in Table 1 [16–20]. These antibodies were purified by a gel filtration chromatography using a Sepharose CL-6B column (Pharmacia, Sweden) for IgM antibodies or by an affinity chromatography using a protein-A column (Bio-Rad Laboratory, CA) for IgG antibody and the purified antibodies were then biotinylated by using sulfo-NHS-biotin (Pierce Chemical Co., IL). An anti-Fas antibody was purchased from MBL (Nagoya, Japan).

### *Induction of apoptosis*

Induction of apoptosis was performed as reported previously [5]. Briefly, $2 \times 10^6$ cells were incubated in a culture bottle with 1000 IU of IFN-$\gamma$ (Chemicon International, Inc., CA) for 24 h. After the removal of IFN-$\gamma$, the cells were incubated with the culture medium containing 500 ng of anti-Fas antibody for 15 h. The cells were used for the following studies after being harvested by trypsin treatment.

### *DNA and RNA extraction and assessment of DNA fragmentation*

$1 \times 10^7$ cells and $1 \times 10^8$ cells were prepared for the extraction of the DNA and the RNA in the cells, respectively. DNA and RNA were extracted by using the DNA extraction kit (Stratagene, CA) and RNA extraction kit (Biotecx Laboratories, Inc., TX) according to the methods instructed in each kit, respectively.

The induction of apoptosis of the cells was confirmed by the appearance of DNA fragmentation in the electrophorogram when 20 $\mu$g of DNA extracted from

**Table 1.** Monoclonal antibodies against carbohydrate antigens

| *Antibody* | *Subclass* | *Determinant* | *Structure* | *Reference* |
|---|---|---|---|---|
| HI-3 | IgM | H | Fuc$\alpha$1,2Gal$\beta$ | 16 |
| FH-2 | IgM | $Le^x$ | Gal$\beta$1,4[Fuc$\alpha$1,3]GlcNAc$\beta$ | 17 |
| BM-1 | IgM | $Le^y$ | Fuc$\alpha$1,2Gal$\beta$1,4[Fuc$\alpha$1,3]GlcNAc$\beta$ | 18 |
| SNH3 | IgM | Sialyl $Le^x$ | NeuAc$\alpha$2,3Gal$\beta$1,4[Fuc$\alpha$1,3]GlcNAc$\beta$ | 19 |
| CF4C4 | $IgG_1$ | $Le^a$ | Gal$\beta$1,3[Fuc$\alpha$1,4]GlcNAc$\beta$ | 20 |

the cells was analysed on a 3% agarose gel stained with ethidium bromide.

*FACS analysis*

$2 \times 10^5$ cells were incubated in an ice bath for 20 min with 1 ml of D-PBS containing 10% goat serum. After washing with cold D-PBS three times, the cells were incubated in an ice bath for 30 min with 1 μg of biotinylated antibody in 100 μl of D-PBS. The cells were washed with cold D-PBS three times and incubated for 30 min in an ice bath with FITC conjugated Streptavidin (Zymed Laboratories, Inc., CA). After washing with cold D-PBS 3 times again, the fluorescent intensity on the cell surface was measured by a flow cytometer (Becton Dickinson, CA).

*Assay protocol for fucosyltransferase activity*

Approximately $1 \times 10^7$ cells were extracted for the enzyme preparation according to our previous method [21]. In order to assay specifically for each enzyme activity in the cell extracts, the specific acceptors, phenyl β-D-galactoside (Sigma Chemical Co., St. Louis, MO), Fucα(1,2)Galβ(1,4)GlcNAcβBn and 2′OMeGalβ(1,3)-GlcNAcβBn (kind gifts from Dr K.L. Matta, Roswell Park Memorial Institute, Buffalo, NY) were used for measuring α(1,2)-, α(1,3)-, and α(1,4)-Fuc T activities, respectively. The assay procedures were performed according to the methods (or the modified methods) in our previous reports [21–23].

*Northern blot analysis of α(1,3)fucosyltransferases*

The 1.7-kilo base pair *Xho* I-*Xba* I fragment was isolated from the insert in pCDM7-α-(1,3/1,4)-Fuc-T [24] and this probe was found to detect Fuc-T V and Fuc-T VI transcripts as well as the Fuc-T III transcript (hence termed the Fuc-T III, V, VI probe). The 591-base pair *Pvu* II-*Ava* II fragment was isolated from the insert in pCDNA1-α-(1,3)-Fuc-T [25] and was used for detecting only Fuc-T IV (hence termed the Fuc-T IV probe). As a specific probe for the Fuc-T VII, the 189-base pair fragment was prepared by PCR from the human Fuc-T VII cDNA [26, 27]. These probes were labelled with $^{32}P$ by a random primer labelling technique and were used for the Northern blot analysis.

Poly(A)$^+$RNAs were purified by applying the total RNA extracts from HT-29 cells cultured with and without anti-Fas antibody to a deoxythymidine oligonucleotide (oligo-dT) cellulose column (Boehringer Mannheim, Tokyo, Japan). Five micrograms of the poly(A)$^+$RNA was fractionated by electrophoresis using a 1.1% agarose gel and transferred to Hybond-N membrane (Amersham, Buckinghamshire, UK). The membrane was pre-hybridized overnight at 42 °C with 0.01 M sodium phosphate buffer, pH 7.7, containing 50% formamide, 0.9 M NaCl, 1 mM EDTA, 0.5% SDS, and 100 μg ml$^{-1}$ denatured herring testis DNA. After the pre-hybridization, each $^{32}P$-probe was added into the pre-hybridization buffer and hybridized overnight at 42 °C. The membrane was washed with 0.015 M trisodium citrate containing 0.3 M NaCl for 10 min at room temperature. After the washing procedure was repeated three times, the membrane was washed with the same buffer containing 0.5% SDS at 60 °C. The RNAs hybridized with the probes were analysed by autoradiography. A $^{32}P$-β-actin probe (Wako pure chemicals, Tokyo, Japan) was used as a control for the amount of the RNA applied on the gel. The density of each band was analyzed by a Video-densitometer (ACI, Tokyo, Japan).

*RT-PCR analysis of α(1,3)fucosyltransferases*

The specific primers [26, 28] listed in Table 2 were prepared for amplifying α(1,3)fucosyltransferases and β-actin mRNA. One microgram of poly(A)$^+$ RNA was pre-treated with DNase I (Takara Shuzo Co Ltd, Kyoto, Japan), the first-strand cDNA synthesis from the RNA was performed with oligo d(T) primer and avian myelomatosis virus reverse transcriptase (Life Sciences, St. Petersburg, FL). The cDNA synthesized was precipitated by ethanol,

**Table 2.** PCR primers for amplifying α(1,3)fucosyltransferese and β-actin mRNA

| *mRNA species detected* | *Primers*[a] |
|---|---|
| Fuc-T III (447-bp) | U: 5′-CTGCTGGTGGCTGTGTGTTTCTTCTCCTAC-3′ |
| and Fuc-T V (486-bp) | L: 5′-CAGCCAGCCGTAGGGCGTGAAGATGTCGGA-3′ |
| Fuc-T IV (319-bp) | U: 5′-GGTGCCCGAAATTGGGCTCCTGCACAC-3′ |
| | L: 5′-CCAGAAGGAGGTGATGTGGACAGCGTA-3′ |
| Fuc-T VI (404-bp) | U: 5′-CTCAAGACGATCCCACTGTGTA-3′ |
| | L: 5′-CAGCCAGCCGTAGGGCGTGAAGATGTCGGA-3′ |
| Fuc-T VII (291-bp) | U: 5′-CTCGGACATCTTTGTGCCCTATG-3′ |
| | L: 5′-CGCCAGAATTTCTCCGTAATGTAG-3′ |
| β-actin (463-bp) | U: 5′-GAGAAGATGACCCAGATCATGT-3′ |
| | L: 5′-ACTCCATGCCCAGGAAGGAAGG-3′ |

[a]U, upper strand primer; L, Lower strand primer.

and the precipitate was dissolved in 20 $\mu$l of 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA. Amplification using 5 $\mu$l of the solution for fucosyltransferase or 1 $\mu$l of the solution for $\beta$-actin (exon 2–5) as a control was performed with 25 pmol of specific primers in the PCR buffer containing 200 $\mu$M each of dNTP, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, and five units of Thermus aquaticus (*Taq*) thermostable DNA polymerase (Cetus, Emeryville, CA). Thirty-five cycles of PCR (1.5 min at 94 °C and 3.5 min at 72 °C) were performed using a Perkin Elmer Cetus DNA thermal cycler. In addition, restriction enzyme digestion was performed using an aliquot of each PCR fragment. The size of the PCR fragments amplified from Fuc-T III and Fuc-T V were 447 bp and 486 bp, respectively. These fragments were not cleavable by *Pvu* II treatment but only the fragment from Fuc-T V was cleaved into two fragments (400 bp and 86 bp) by the *Nae* I treatment. The fragments amplified from Fuc-T IV (319 bp) and Fuc-T VI (404 bp) were cleaved into two fragments (176-, 143-bp and 323-, 81-bp, respectively) by *Apa* I and *Pvu* II treatments, respectively. An aliquot of each reaction was electrophoresed on Tris-borate/EDTA-buffered 5% polyacrylamide gel. The same experimental procedure without reverse transcriptase was performed and no amplification derived from contamination by genomic DNA was ascertained.

## Results

*DNA fragmentation in anti-Fas treated cells*

As shown in Fig. 1, ladder formation of DNAs extracted from HT-29 cells was observed on the electropherogram when the cells were successively treated with IFN-$\gamma$ and anti-Fas antibody. No DNA fragmentation was observed when the cells were treated only with IFN-$\gamma$ but treatment by anti-Fas antibody produced a little DNA fragmentation (data not shown). Therefore, cells treated with both IFN-$\gamma$ and anti-Fas antibody were considered as undergoing the process of apoptosis.

*FACS analysis of apoptotic cells*

Changes of the expression of carbohydrate antigens on the HT-29 cell surface associated with apoptosis were examined by FACS analysis. As shown in Fig. 2, a strongly enhanced expression of $Le^x$ and a slightly enhanced expression of $Le^y$ and H antigens were observed on the cell surface of the treated HT-29 cells. The ratios of mean fluorescence intensities (mean fluorescence intensity on the treated cells/mean fluorescence intensity on the untreated cells) of $Le^x$, $Le^y$ and H antigens were calculated as 4.5, 1.6 and 1.3, respectively. On the other hand, no such increase of expression was observed for the other carbohydrate antigens tested, and the ratios for the $Le^a$ and sialyl $Le^x$ antigens were calculated to be 0.9 and 0.8,

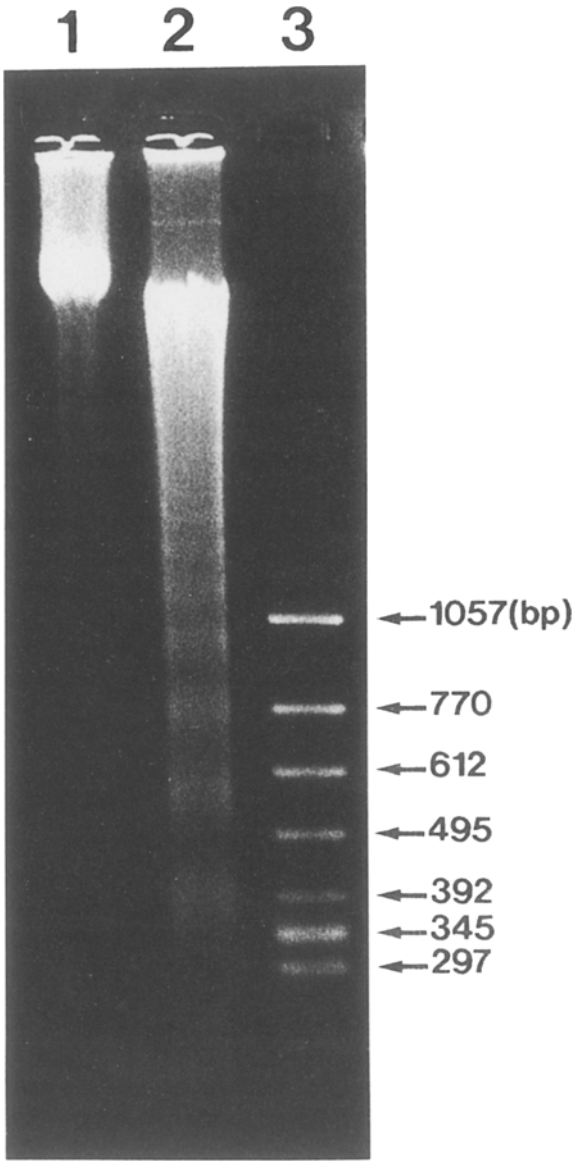


**Figure 1.** Fas engagement results in DNA fragmentation in HT-29 cells. Genomic DNA extracted from HT-29 cells cultured without (lane 1) and with (lane 2) anti-Fas antibody and the molecular marker (lane 3).

respectively. This result suggests that the effect of exogenous neuraminidase should be minimal, since the increase of $Le^x$ antigen expression by treatment of the cells with neuraminidase usually accompanies a marked decrease of sialyl $Le^x$ antigen expression. This led us to study changes in fucosyltransferase activities of the treated cells.

*Changes in fucosyltransferase activities*

$\alpha$(1,2)-, $\alpha$(1,3)- and $\alpha$(1,4)-Fuc-T activities in the HT-29 cell lysates were examined using specific acceptors for each enzyme. In this study, H type II trisaccharide was

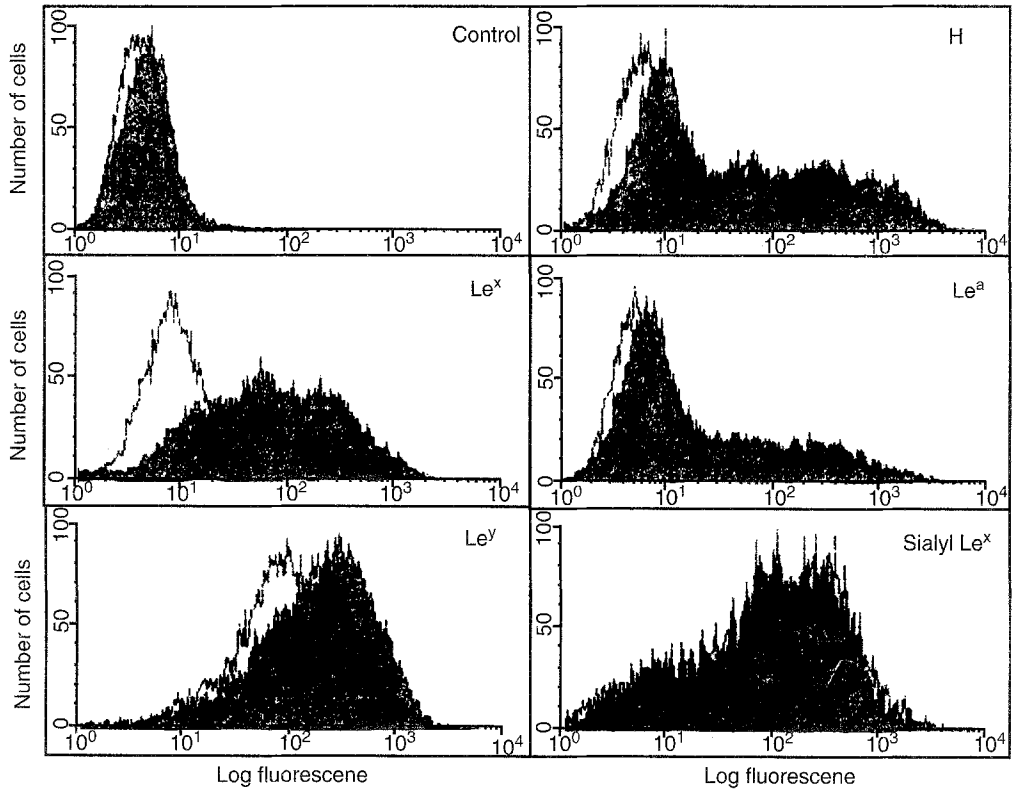


**Figure 2.** Fluorescence histograms of HT-29 cells in FACS analysis. Filled histograms express the fluorescence intensity on the surface of the cells cultured with anti-Fas antibody and unfilled histograms express that on the surface of the cells cultured without the antibody. The control histogram was obtained by using the unrelated antibody instead of antibodies against carbohydrate antigens.

used as the acceptor for α(1,3)-Fuc T, since this structure was reported to serve as a better acceptor for Fuc-T IV than *N*-Acetyllactosamine [29, 30]. As shown in Fig. 3, the α(1,3)-Fuc-T activity in the lysate of the cells treated with IFN-γ and anti-Fas antibody was obviously elevated compared to that in the lysate of the untreated cells. On the other hand, α(1,2)-Fuc-T activity was found to be slightly elevated and the elevation of α(1,4)-Fuc-T activity was very limited after the treatment. The results suggests that the increased expression of the fucosylated antigens, in particular of the $Le^x$ antigen in the treated cells, seems to result from the elevated level of α(1,3)-Fuc-T activity.

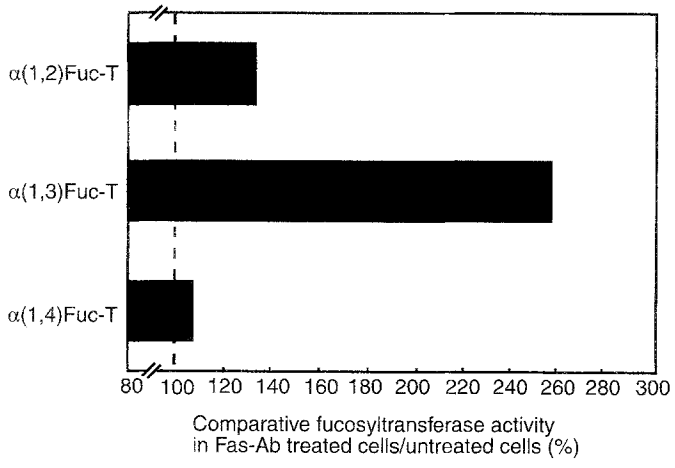


**Figure 3.** Comparative fucosyltransferase activities in HT-29 cell lysate treated with anti-Fas antibody. The ratios of the enzyme activities were calculated by the following formula; enzyme activity in the treated cell lysate/enzyme activity in the untreated cell lysate (%). The specific acceptors for each enzyme activity were used (see Materials and Methods).

*Northern blot and RT-PCR analyses of α(1,3)-Fuc-T*

As described previously [28], the Fuc-T III, V, VI probe and the Fuc-T IV probe were shown to detect transcripts in A431 (Fig. 4, lane 1) and HL-60 (Fig. 4, lane 2), respectively. In the untreated HT-29 cells, the 2.3 kb RNA message for Fuc-T III, V and/or VI was clearly detected, while the RNA message specific for Fuc-T IV was only faintly detected (Fig. 4, left and middle panels, lane 3). In the cells treated with IFN-γ and anti-Fas antibody, however, the RNA message for Fuc-T III, V, and/or VI was remarkably decreased. The message for Fuc-T IV was found to be obviously increased and it corresponded to the 2.3 kb mRNA (Fig. 4, left and middle panels, lane 4). The message level of Fuc-T III was shown to be extremely decreased and that of Fuc-T IV increased to 2.8-fold after treatment with anti-Fas antibody (Fig. 5). The RNA message for Fuc-T VII was hardly detectable in either

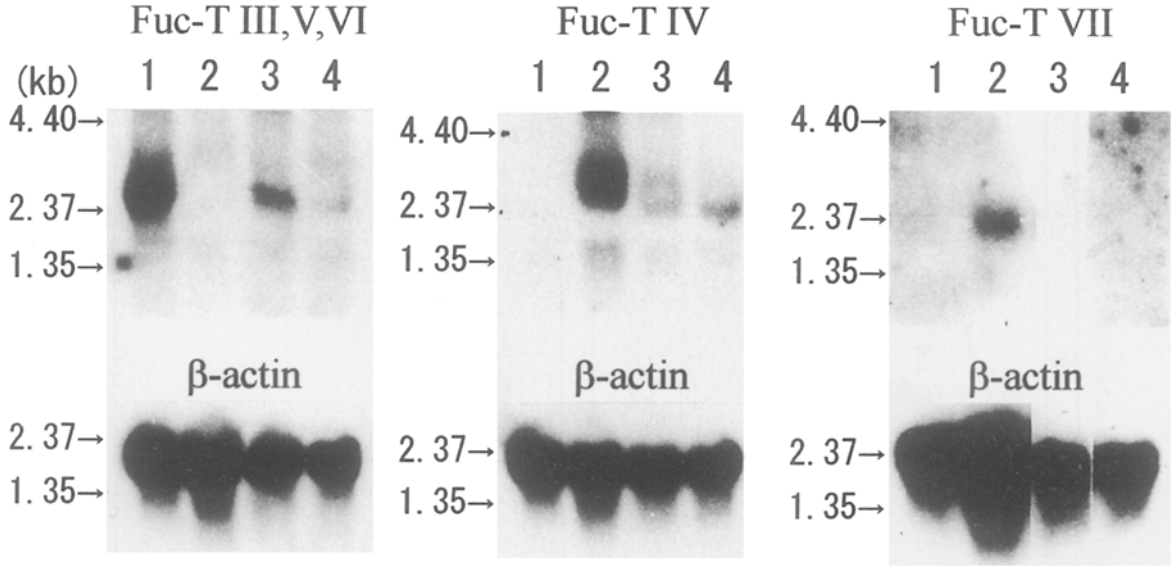


**Figure 4.** Northern blot analysis of HT-29 cells. Poly(A)$^+$ RNA (5 $\mu$g each) extracted from A431 cells (lane 1), HL-60 cells (lane 2) and untreated (lane 3) and treated (lane 4) HT-29 cells were electrophoresed, blotted, and hybridized with the $^{32}$P-labelled Fuc-T III, V, VI probe, Fuc-T IV probe, Fuc-T VII probe, and $\beta$-actin probe and then were autoradiographed as described in Materials and Methods. Left of each picture, sizes of each band indicated in kilobases (*kb*).

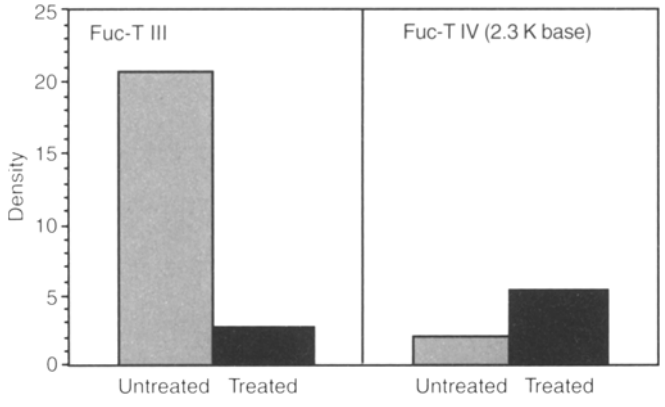


**Figure 5.** Changes of message levels in Fuc-T III (left panel) and Fuc-T IV (right panel) from HT-29 treated with anti-Fas antibody. Density of the bands in Fig. 4 was analysed by a densitometer.

treated or untreated cells (Fig. 4, right panel, lanes 3 and 4).

In order to discriminate the messages of fucosyltransferases detected by the Fuc-T III, V, VI probe, and to detect a smaller quantity of transcripts which had probably been below the detection level in the Northern blot analysis, RT-PCR analysis was performed using specific primers for each fucosyltransferase (Fig. 6). No amplification was observed for Fuc-T V but a clear 447 bp fragment which was not cleaved by the *Pvu* II digestion was amplified from Fuc-T III. A weak 404 bp fragment was amplified from Fuc-T VI which was cleaved into two fragments, 323- and 81-bp by the *Pvu* II digestion. These results therefore suggested that the strong message in the untreated cells detected by the Fuc-T III, V, VI probe in the Northern blot analysis was a mixture of a large amount of Fuc-T III and a small amount of Fuc-T VI. A 319 bp fragment was amplified from Fuc-T IV and was cleaved into two fragments, 176- and 143-bp by the *Apa* I treatment. The RNA message for Fuc-T IV detected in the Northern blot analysis was reconfirmed in the RT-PCR analysis. A specific amplification for Fuc-T VII was faintly detectable before and after treatment with IFN-$\gamma$ and anti-Fas antibody.

## Discussion

In this study, changes in both the expression of fucosylated carbohydrate antigens and the activities of $\alpha$(1,2)-, $\alpha$(1,3)- and $\alpha$(1,4)-Fuc-Ts were investigated in the human colon adenocarcinoma HT-29 cells in the process of apoptosis caused by treatment with IFN-$\gamma$ and anti-Fas antibody. In addition, changes of the levels of RNA messages for Fuc-T III, IV, V, VI and VII genes were also analysed. In the FACS analysis, a strongly enhanced expression of Le$^x$ and slightly enhanced expressions of H and Le$^y$ antigens were found on the cell surface. On the other hand, a slightly decreased expression of Le$^a$ and sialyl Le$^x$ antigens was observed on the same cells. The results seemed to be consistent with those of the fucosyltransferase assay showing a significant elevation of $\alpha$(1,3)-Fuc-T activity with a slight and no elevation of

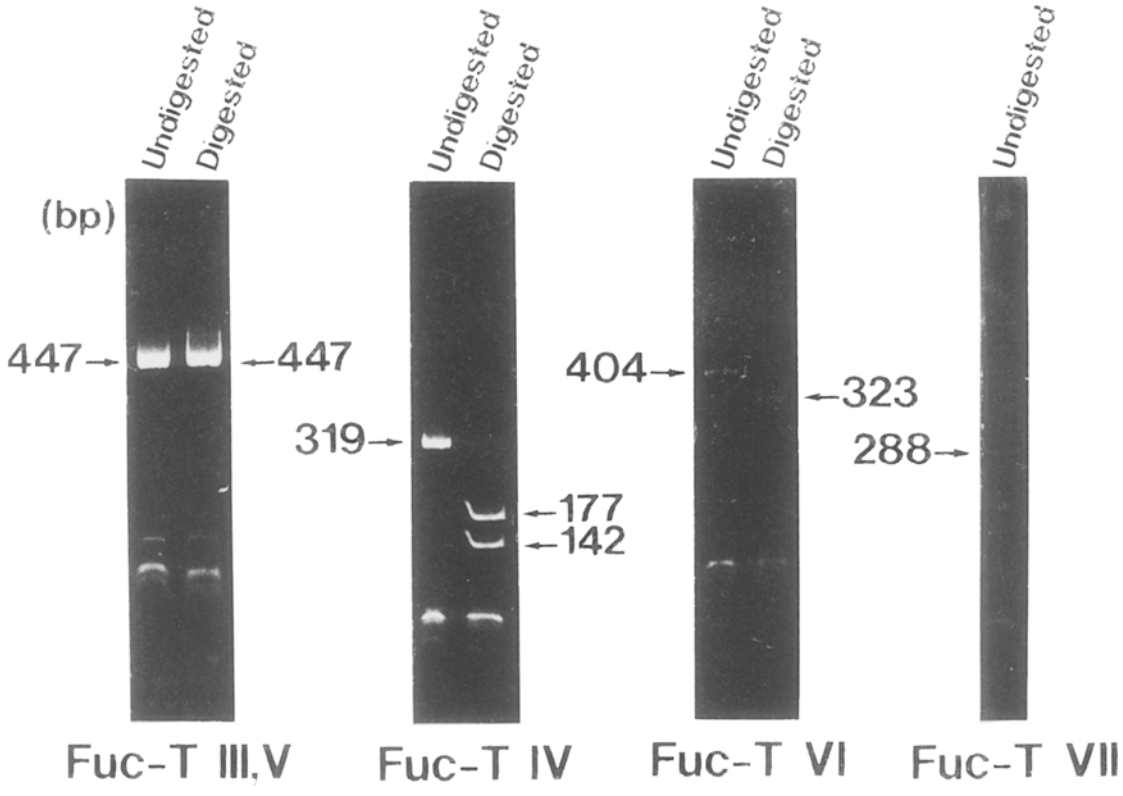


**Figure 6.** RT-PCR analysis of HT-29 cells. An aliquot of PCR products was digested with restriction enzymes as described in Materials and Methods and both the undigested and the digested fragments were electrophoresed in 5% polyacrylamide gel and then stained with ethidium bromide. Right, sizes of digested PCR fragments. Left, sizes of undigested PCR fragment.

α(1,2)- and α(1,4)-Fuc-T activity, respectively. It was reported in a previous immunohistochemical study that $Le^y$ antigen expression correlated with apoptosis in cancer tissues [11] and $Le^y$ antigen expression was concluded to be a useful phenotypic marker of apoptosis. In the present study, however, an inconsistent result showing that the expression of $Le^x$ antigen was more enhanced than that of $Le^y$ antigen was obtained even though the $Le^y$ antigen was observed to be highly expressed in comparison with the $Le^x$ antigen on the cell surface of the untreated cells.

Recently, five Fuc-T genes (Fuc-T III, IV, V, VI and VII) encoding α(1,3)-Fuc-Ts have been isolated and cloned [24–28, 31, 32]. The Fuc-T III, V and VI genes were found to locate on the chromosome 19 and only the Fuc-T III gene was demonstrated to encode both α(1,3)- and α(1,4)-Fuc-Ts. The cDNA sequences of the catalytic domain from each fucosyltransferase were shown to have more than 90% homology to each other. On the other hand, the Fuc-T IV gene was found to locate on the chromosome 11 and the cDNA sequence of the catalytic domain showed 70–73% homology compared with that of Fuc-T III, V or VI. Furthermore, the Fuc-T VII gene was found to locate on the chromosome 9 showing only 30–40% homology in the cDNA sequence compared to the sequences of other Fuc-Ts. In this study, RNA message levels of these Fuc-Ts were examined by a Northern blot technique using three probes, i.e., a probe common for Fuc-T III, V, VI whose sequences are highly homologous to each other and two specific probes for Fuc-T IV and VII, respectively. It was clearly demonstrated that the RNA message level for Fuc-T IV increased and that for Fuc-T III, V, VI decreased in the HT-29 cells treated with IFN-γ and anti-Fas antibody. It was reported that three species of the message, two major bands with 2.3 and 6.0 kbs and one minor one with 3.0 kb, were detected in Northern blot analysis for Fuc-T IV [33]. In the present study, however, only two species of the message with 2.3 and 3.0 kbs which must correspond to both FLFT and ELFT-L, respectively [33] were faintly detected in untreated HT-29 cells. On the other hand, the message with 6.0 kb disappeared by the enhancement of hybridization stringency (data not shown) and the message seemed to be derived from homologous genes other than the Fuc-T IV gene. Interestingly, only the message with 2.3 kb was demonstrated to increase in the HT-29 cells treated with IFN-γ and anti-Fas antibody. Although the message with 3.0 kb has no consensus sequence required for translation initiation [34], it has also been reported

that the ELFT-L transfected cells produce the ELFT-like antigen [33] and that the message was detected in various cancer cell lines [28]. Whether the level of $\alpha$(1,3)-Fuc-T activity in the treated HT-29 cells was elevated only due to the increased level of the RNA message with 2.3 kb in Fuc-T IV remains to be investigated. However, the present results strongly indicated that the increased message for Fuc-T IV must be responsible for the activation of the $\alpha$(1,3)-Fuc-T gene resulting in the increases of both $\alpha$(1,3)-Fuc-T activity and $Le^x$ antigen expression associated with apoptosis.

Although the physiological functions of fucosylated carbohydrate antigens have not been known well, a stage-specific embryonic antigen (SSEA-1) whose determinant was demonstrated to be a series of carbohydrate antigens carrying $Le^x$ hapten, Gal$\beta$1,4[Fuc$\alpha$1,3]GlcNAc$\beta$, was found on the cell surface of murine embryos beginning at the 8-cell stage and considered to be one of the differentiation antigens [17, 35, 36]. The SSEA-I antigen family, such as $Le^x$ and $Le^y$ antigens, has been shown to be expressed on the blastocyte surface and on the developing gland cells during the formation of bronchial glands in human lung embryos [37, 39, 40]. The expression of $Le^x$ and $Le^y$ antigens has also been known to be related with oncogenesis [40–42]. $\alpha$(1,3)-Fuc-T activities responsible for the synthesis of the SSEA-1 antigen family were found in human sera and the elevated activity of the enzyme has been demonstrated in serum from patients with various cancers [22, 42]. In addition, elevated $\alpha$(1,2)-Fuc-T activities responsible for the synthesis of H and $Le^y$ as well as $Le^b$ antigens was demonstrated in colorectal cancer tissues [31, 43, 44], and also $Le^x$ and $Le^y$ determinants were demonstrated to play a role in cell-to-cell adhesion via homotypic ($Le^x$-$Le^x$) or heterotypic ($Le^x$-$Le^y$) interactions [38, 40]. The functional mechanism which links $\alpha$(1,3)-fucosylation to apoptosis remains unknown at this time, but the accumulation of $Le^x$ antigen in cells undergoing the apoptotic process observed in this study might have some significance in the altered cell-to-cell interaction or adhesion between target cells and cytotoxic killer T-cells in the process of apoptosis.

In summary, the expression of $Le^x$ antigen on the cell surface was strongly enhanced in accordance with the appearance of apoptotic cell death when HT-29 cells were treated sequentially with IFN-$\gamma$ and anti-Fas antibody. The enhancement of $Le^x$ antigen expression was assumed to result from the elevated level of $\alpha$(1,3)-Fuc-T activity mainly due to the increased level of the RNA message for the Fuc-T IV gene in such cells.

## Acknowledgement

We are grateful to Dr Sen-itiroh Hakomori, The Biomembrane Institute and Department of Pathology, University of Washington, Seattle, Washington, for the gifts of antibodies directed to carbohydrate antigens, to Dr John B. Lowe, Howard Hughes Medical Institute, University of Michigan Medical School, Ann Arbor, Michigan, for the gifts of genetic probes for fucosyltransferases, and to Dr Khushi L. Matta, Roswell Park Memorial Institute, Buffalo, NY, for the gifts of carbohydrate antigens.

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